REMARKS

The Examiner has required election of one of the groups identified by the Examiner on pages 2 and 3 of the Office Action.

In support of the Restriction Requirement, the Examiner has stated that the various inventions do not relate to a single inventive concept, because they lack the same or corresponding technical features. According to the Examiner, the technical feature linking Groups A-J appears to be that they all relate to a nitrile hydratase comprising variants of SEQ ID NO: 1 and/or SEQ ID NO: 2. However, because a nitrile hydratase comprising an α and β subunit wherein the α subunit is substituted at positions 37, 71, and 148 of SEQ ID NO: 1 is allegedly described in Kobayashi et al., Biochimica et Biophysica Acta 1129:23-33, 1991, the Examiner has concluded that the reference sequence anticipates the present claims. Accordingly, the Examiner has concluded that the invention as recited does not define a contribution over the prior art and thereby fails to constitute a special technical feature.

In response to the Restriction Requirement, Applicants hereby elect with traverse Group C, defined by the Examiner as a nitrile hydratase which comprises variants of the α and β subunits of SEQ ID NO: 1 and 2, respectively, and a method of use of said nitrile hydratase for producing an amide compound. The reasons for traversal are as follows:

Applicants further submit that Kobayashi et al. does not teach or even suggest the subject matter of the present claims. Thus, the present invention defines a contribution over the prior art and thereby constitutes a special technical feature which confers unity of invention.

In particular, Kobayashi et al. discloses two kinds of cobalt-containing nitrile hydratases (NHases) referred to as J1-H and J1-L and produced by *Rhodococcus rhodochrous*

J1; one NHase from *Rhodococcus sp.* N-774; amino acid sequences thereof; and base sequences of the genes coding for the NHases. At page 30, FIG. 4, of Kobayashiet et al., amino acid sequences for those three kinds of NHases are mentioned. In FIG. 4, the sequences are aligned with the N-terminal shift appropriate for demonstrating the homologies of the NHase sequences. A simple comparison of the 37th position from the N-terminal of J1-L and J1-H shows that "Thr" is changed to "Val". Further, at the 71st position from the N-terminal of N-774 to J1-H, "Arg" is changed to "Thr". At the 148th position from the N-terminal of N-774 to J1-H, "Gly" is changed to "Glu".

Applicants have enclosed a number of sequence alignments for the Examiner's reference. In the alignments, "P.T.—A" refers to alpha—subunit of the nitrile hydratase from Pseudonocaldia Thermophira (SEQ ID NO. 1 of the present application); "P.T-B" to beta-subunit of the nitrile hydratase from Pseudonocaldia Therrnophira (SEQ ID NO. 2 of the present application); "R.R-J1-HA" to alpha-subunit of J1-H from Rhodococcus rhodochrous; "R.R-J1-HB" to beta-subunit of J1-H from Rhodococcus rhoclochrous; "R.R-J1-LA" to alpha-subunit of J1-L from Rhodococcus rhodochrous; and "R.R-J1-LB" to beta-subunit of J1-L from Rhodococcus rhodochrous.

As shown by the attached alignments, the homology of the NHases from the J1 strain and *Pseudonocaldia Thermophira* (the present invention) are 43 % based on comparison of J1-H with *Pseudonocaldia Therrnophira*, and 52 % based on comparison of J1-L with *Pseudonocaldia Thermophira*. Therefore, both of the reference NHases are totally different enzymes as compared to the inventive enzyme. Further, both enzymes are derived from different organism species. Therefore, even if the substitutions of amino acid sequences for the enzyme of Kobayashi et al. are made at the amino acid positions 37, 71 and 148 amino acids from N-terminal to the same amino acids as the present invention (that is, if the

substitutions corresponding to the present invention were made on the sequences for the enzymes of Kobayashi et al.), the other part of those sequences for the NHases of Kobayashi et al. are still different from that of the present invention. Accordingly, the resulting amino acid sequences for the NHases of Kobayashi et al. and the invention are totally different. Applicants submit that the Examiner's position that the present invention lacks novelty as being anticipated by Kobayashi et al. is not correct.

Applicants further note that the Examiner's comparison is based not on comparison between before and after substitution of "the amino acid sequences for the basic enzyme" (with respect to the present invention, sequences of SEQ ID NO. 1 and/or SEQ ID NO. 2), but on comparison between the amino acids at the position by the number corresponding to the substitution of the invention from the N-terminal of three kinds of the native NHases. Based on this unreasonable comparison, the Examiner maintains that similar alternation is involved in Kobayashi et al. and the present invention.

However, Kobayashi et al. never discloses that the new amino acid sequence is obtained by substituting amino acids in the sequence for the basic enzyme for the other amino acids to obtain the new modified enzyme. Therefore, technical field of Kobayashi et al. is different from that of the present invention. Furthermore, in a manner of the comparison employed by the Examiner, a frame showing the homology described in Kobayashi et al. was ignored, and then amino acid residue of the sequences at the position by the number corresponding to the substitution of the invention from the N-terminal were compared. It is important for the one skilled in the art that the sequences should be aligned, before both amino acid sequences are compared, since such a proper comparison provide information regarding the significant part of the sequence, such as an enzymatic active center. In contrast, the Examiner's comparison is focused on amino acids at the position that would be

acknowledged by the one of skill in the art not to provide functionally homology for the enzyme molecule.

It is in accepted in this technical field that a new modified enzyme will be generated by altering one or more amino acids at the position showing functionally homology for the enzyme molecule or being related structurally and/or chemically to the sequence for the basic enzyme. The Examiner merely indicates in the Action that the same amino acid residue as substituted in the invention is described when comparing amino acids of two different enzymes (both are also different from the enzyme of the invention) at the position relative to the N-terminal corresponding to the substitution of the invention. Therefore, when the Examiner's comparison is applied to an basic NHase for the invention, which is derived from a different organism species and has a different amino acid sequence, it is not suggested whether the enzymatic activity will be improved or not, even whether the enzymatic activity will be maintained or not.

In view of the above, Applicants respectfully request reconsideration and withdrawal of the Restriction Requirement.

CONCLUSION

This response is made without prejudice or disclaimer to any non-elected subject matter, and Applicants reserve the right to file one or more continuation and/or divisional applications directed to any non-elected subject matter.

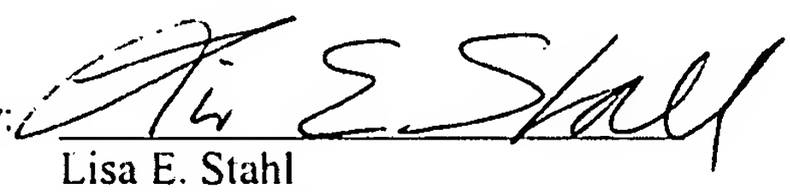
In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions related to this response, or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney at the below-listed telephone number concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

Date: January 28, 2008

By: 
Lisa E. Stahl
Registration No. 56704

P.O. Box 1404
Alexandria, VA 22313-1404
703 836 6620

```
CLUSTAL W (1.81) Multiple Sequence Alignments


Sequence type explicitly set to Protein
Sequence format is Pearson
Sequence 1: P.T.-A          205 aa
Sequence 2: R.R-J1-HA       203 aa
Start of Pairwise alignments
Aligning...


Sequences (1:2) Aligned. Score: 53.6946
Sequences (2:2) Aligned. Score: 100
Guide tree        file created:   [clustalw.dnd]
Start of Multiple Alignment
There are 1 groups
Aligning...
  oup 1: Sequences:   2      Score:1743
Alignment Score 756
CLUSTAL-Alignment file created  [clustalw.aln]
```

clustalw.aln

```
CLUSTAL W (1.81) multiple sequence alignment

P.T.-A          MTENILRKSDEEIQKEITARVKALESMLIEQGILTTSMIDRMAEIYENEVGPHLGAKVVV
R.R-J1-HA       MSEHVNKYT------EYEARTKAIETLLYERGLITPAAVDRVQSYYENEIGPMGGAKVVA
                *:*:: : :      *  **.**:*::* *:*::*.:.:**:.. ****:**  *****.

P.T.-A          KAWTDPEFKKSLLADGTEACKELGIGGLQGEDMMWVENTDEVHHVVVCTLCSCYPWPVLG
R.R-J1-HA       KSWVDPEYRKWLEEDATAAMASLGYAGEQAHQISAVFNDSQTHHVVVCTLCSCYPWPVLG
                *:*.***::* *  *.* *  .** .* *..::  * * .:.******************

P.T.-A          LPPNWFKEPQYRSRVVREPRQLLKEEFGEEVPPSKEIKVWDSSSEMRFVVLPQRPAGTDG
R.R-J1-HA       LPPAWYKSMEYRSRVVADPRGVLKRDFGFDIPDSVEVRVWDSSSEIRYIVIPERPAGTDG
                *** *:*. :******* :** :**.:***::* . *:*******:*::*:*:*******

P.T.-A          WSEEELATLVTRESMIGVEPAKAVA----
  R-J1-HA       WSEEELTKLVSRDSMIGVSNALTPQEVIV
                ******:.**:*:*****. * :
```

clustalw.dnd

```
(P.T.-A:0.23153,R.R-J1-HA:0.23153);
```

Select tree menu Exec

Generate profile HMM

```
 CLUSTAL W (1.81) Multiple Sequence Alignments


Sequence type explicitly set to Protein
Sequence format is Pearson
Sequence 1: P.T.-B          233 aa
Sequence 2: R.R-J1-HB       225 aa
Start of Pairwise alignments
Aligning...


Sequences (1:2) Aligned. Score: 31.5556
Sequences (2:2) Aligned. Score: 100
Guide tree        file created:   [clustalw.dnd]
Start of Multiple Alignment
There are 1 groups
Aligning...
Group 1: Sequences:   2      Score:1541
Alignment Score 443
CLUSTAL-Alignment file created  [clustalw.aln]
```

clustalw.aln

```
CLUSTAL W (1.81) multiple sequence alignment

P.T.-B          MNGVYDVGGTDGLGPINRPADEPVFRAEWEKVAFAMFPATFRAGFMGLDEFRFGIEQMNP
R.R-J1-HB       MDGIHDTGGMTGYGPVPYQKDEPFFHYEWEGRTLSILTWMHLKGISWWDKSRFFRESMGN
                *.*::*.**  * **:    ***.*: ***  ::::::.  .  *:  *: ** .*.*.

P.T.-B          AEYLESP---YYWHWIRTYIHHGVRTGKIDLEELERRTQYYRENPDAP-LPEHEQKPELI
R.R-J1-HB       ENYVNEIRNSYYTHWLSAAERILVADKIITEEERKHRVQEILEGRYTDRKPSRKFDPAQI
                 :*:.:.    ** **: :  :  *    *  ** ::*.*   *. :  *.:: .*  *

P.T.-B          EFVNQAVYGGLPASREVDRPPKFKEGDVVRFSTASPKGHARRARYVRGKTGTVVKHHGAY
R.R-J1-HB       EKAIERLHEPHSLALPG-AEPSFSLGDKIKVKSMNPLGHTRCPKYVRNKIGEIVAYHGCQ
                *  . : ::   . :      *.*. ** ::..: .* **:* .:***.* * :* :**.

P.T.-B          IYPDTAGNGLGECPEHLYTVRFTAQELWGPEGDPNSSVYYDCWEPYIELVDTKAAAA
R.R-J1-HB       IYPESSSAGLGDDPRPLYTVAFSAQELWGDDGNGKDVVCVDLWEPY----------
                ***:::. ***: *: **** *:****** :*: :. *  * ****
```

clustalw.dnd

```
(P.T.-B:0.34222,R.R-J1-HB:0.34222);
```

Select tree menu Exec

Generate profile HMM

```
CLUSTAL W (1.81) Multiple Sequence Alignments


Sequence type explicitly set to Protein
Sequence format is Pearson
Sequence 1: P.T.-A          205 aa
Sequence 2: R.R-J1-LA       207 aa
Start of Pairwise alignments
Aligning...


Sequences (1:2) Aligned. Score: 55.6098
Sequences (2:2) Aligned. Score: 100
Guide tree        file created:   [clustalw.dnd]
Start of Multiple Alignment
There are 1 groups
Aligning...
  oup 1: Sequences:   2      Score:1813
Alignment Score 786
CLUSTAL-Alignment file created  [clustalw.aln]
```

clustalw.aln

```
CLUSTAL W (1.81) multiple sequence alignment

P.T.-A          -MTENILRKSDEEIQKEITARVKALESMLIEQGILTTSMIDRMAEIYENEVGPHLGAKVV
R.R-J1-LA       MTAHNPVQGTLPRSNEEIAARVKAMEAILVDKGLISQDAIDHMSSVYENEVGPQLGAKIV
                  :.* :: :  . ::**:*****:*::*:::*:::*. **:*:.:*******:****:*

P.T.-A          VKAWTDPEFKKQLLADGTEACKELGIGGLQGEDMMWVENTDEVHHVVVCTLCSCYPWPVL
R.R-J1-LA       ARAWVDPEFKQRLLTDATSACREMGVGGMQGEEMVVLENTGTVHNMVVCTLCSCYPWPVL
                .:**.*****:***:*.*.**:*:*:**:***:*: :***. **::***************

P.T.-A          GLPPNWFKEPQYRSRVVREPRQLLKEEFGFEVPPSKEIKVWDSSSEMRFVVLPQRPAGTD
R.R-J1-LA       GLPPNWYKYPAYRARAVRDPR-GVLAEFGYTPDPDVEIRIWDSSAELRYWVLPQRPAGTE
                ******:* * **:*.**:**   :  ***:   *. **::****:*:*: *********:

P.T.-A          GWSEEELATLVTRESMIGVEPAKAVA--
R.R-J1-LA       NFTEEQLADLVTRDSLIGVSVPTTPSKA
                .::**:** ****:*:***. ..: :
```

clustalw.dnd

```
(P.T.-A:0.22195,R.R-J1-LA:0.22195);
```

Select tree menu Exec

Generate profile HMM

```
 CLUSTAL W (1.81) Multiple Sequence Alignments


Sequence type explicitly set to Protein
Sequence format is Pearson
Sequence 1: P.T.-B          233 aa
Sequence 2: R.R-J1-LB       225 aa
Start of Pairwise alignments
Aligning...


Sequences (1:2) Aligned. Score: 46.6667
Sequences (2:2) Aligned. Score: 100
Guide tree        file created:   [clustalw.dnd]
Start of Multiple Alignment
There are 1 groups
Aligning...
Group 1: Sequences:   2      Score:1931
Alignment Score 734
CLUSTAL-Alignment file created  [clustalw.aln]
```

clustalw.aln

```
CLUSTAL W (1.81) multiple sequence alignment

P.T.-B          MNGVYDVGGTDGLGPINRPADEPVFRAEWEKVAFAMFPATFRAGFMGLDEFRFGIEQMNP
R.R-J1-LB       MDGIHDLGGRAGLGPIKPESDEPVFHSDWERSVLTMFPAMALAGAFNLDQFRGAMEQIPP
                *:*::*:**  *****:  :*****:::**: .::****   ** :.**:** .:**: *

P.T.-B          AEYLESPYYWHWIRTYIHHGVRTGKIDLEELERRTQYYRENPDAPLPEHEQKPELIEFVN
R.R-J1-LB       HDYLTSQYYEHWMHAMIHHGIEAGIFDSDELDRRTQYYMDHPDDTTPTR-QDPQLVETIS
                 :** * ** **::: ****:.:* :* :**:****** ::** . * : *.*:*:* :.

P.T.-B          QAVYGGLPASREVDRPPKFKEGD-VVRFSTASPKGHARRARYVRGKTGTVVKHHGAYIYP
R.R-J1-LB       QLITHGADYRRPTDTEAAFAVGDKVIVRSDASPNTHTRRAGYVRGRVGEVVATHGAYVFP
                * : *   * .* . * ** *:  * ***: *:*** **** :* **  ****::*

P.T.-B          DTAGNGLGECPEHLYTVRFTAQELWGPEGDPNSSVYYDCWEPYIELVDTKAAAA
R.R-J1-LB       DTNALGAGESPEHLYTVRFSATELWGEPAAPNVVNHIDVFEPYLLP--------
                ** . * ** *********:* ****  . **   : * :***:
```

clustalw.dnd

```
(P.T.-B:0.26667,R.R-J1-LB:0.26667);
```

Select tree menu Exec

Generate profile HMM